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Non-Alcoholic Fatty Liver Disease Is Strongly Associated with Smoking Status and Is Improved by Smoking Cessation in Japanese Males: A Retrospective Study

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BACKGROUND: Cigarette smoking is known to be a significant risk factor associated with non-alcoholic fatty liver disease (NAFLD). We aimed to examine the association between smoking status and the severity of fatty liver with regard to sex and smoking cessation. METHODS: In total, 13,466 subjects (6,642 males and 6,824 females) who had undergone abdominal ultrasonography for health check-up, multivariable logistic regression analysis was retrospectively conducted to assess the association between smoking status and the prevalence of NAFLD stratified by sex after adjusting for other potential confounders. RESULTS: Male sex (odds ratio [OR] 3.27, 95% confidence interval [CI] 3.00-3.57) and smoking history (former smoker: OR 1.23, 95% CI 1.10-1.38, current smoker: OR 1.31, 95% CI 1.17-1.47) were significantly associated with NAFLD. In males with a smoking history, an increased pack-year was strongly associated with the prevalence and severity of NAFLD (prevalence of moderate to severe fatty liver: Pack-year from 0.01 to 9.99, 21.3%; Pack-year from 10.00 to 19.99, 27.2%; Pack-year ≥20.00, 33.7%; P<0.0001), although the prevalence of moderate to severe fatty liver was inversely associated with the duration of smoking cessation (more than 10 years vs. within 5 years, OR 0.71, 95% CI 0.53-0.96). In female subjects, light current smoking was negatively associated with NAFLD (current smoker with a pack-year from 0.01 to 9.99 vs. never smoker, OR 0.41, 95% CI 0.19-0.76). CONCLUSIONS: Smoking status and pack-year were strongly associated with the prevalence and severity of NAFLD, especially in Japanese males. However, smoking cessation improved NAFLD in this population.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a term used to describe chronic liver diseases encompassing the entire spectrum of fatty liver disease from fatty liver to steatohepatitis and cirrhosis in individuals without significant alcohol consumption[1]. The prevalence of NAFLD has increased worldwide, including in Japan, along with the increase in obesity and metabolic syndrome[2-4]. Recent studies from the United States have reported that liver-related mortality was increased in association with the increased prevalence of NAFLD[5, 6].

Major risk factors of NAFLD are obesity and type 2 diabetes mellitus, but several studies have shown that cigarette smoking is associated with the onset of NAFLD[7, 8, 9]. A recent meta-analysis on the association between smoking and NAFLD reported that previous smoking was significantly associated with NAFLD, although there was no significant association between current smoking and NAFLD[10].

However, whether the association between smoking status and NAFLD differs by gender has not been determined. In a previous study, only male subjects were included because the number of female active smoker was too small to evaluate statistically[11]. In order to clarify the difference by gender, a cross-sectional study examining the association between smoking history and NAFLD was carried out.

MATERIALS AND METHODS

Subjects

We enrolled 17,915 subjects who had undergone a health check-up, including abdominal ultrasonography, at Yodogawa Christian Hospital between January and December 2016. At their health check-ups, all subjects were asked to complete a questionnaire about their medical histories, prescribed medications, alcohol consumption, and

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smoking histories. Subjects who had no data of hepatitis B virus surface antigen (HBsAg) or hepatitis C virus antibody (HCV Ab) (n=802), those who tested positive for HBsAg (n=160), those who tested positive for HCV Ab (n=144), and those who had incomplete information on smoking history (n=961) were excluded. Subjects whose alcohol consumption was greater than 30 g/day in males (n=1,747) and 20 g/day in females (n=635) were also excluded. The remaining 13,466 subjects were analysed in this study.

This study was conducted in accordance with the Declaration of Helsinki and its amendments. The study protocol was approved by the ethics committee of Yodogawa Christian Hospital.

Definition

Abdominal ultrasonography of the subjects was performed by trained technicians, and the findings of the abdominal ultrasonography were evaluated by gastroenterologists. Fatty liver was diagnosed based on the abdominal ultrasonography findings when hepatorenal echo contrast and liver brightness were observed. The subjects with vascular blurring along with liver brightness were classified as subjects with moderate to severe fatty liver. Body mass index (BMI) was calculated by dividing body weight (kg) by the height squared (m^2), and obesity was defined as a BMI ≥25. For alcohol consumption, the subjects were classified into two groups: non-drinkers (0 g/day) and light drinkers (less than 30 g/day in men and 20 g/day in women). The subjects with diabetes mellitus were defined as those with fasting plasma glucose ≥126 mg/dl and HbA1c ≥6.5% or those who were receiving drug treatment for diabetes mellitus. The subjects with hypertension were defined as those with a systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or those who were receiving drug treatment for hypertension. The subjects with dyslipidaemia were defined as those with low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dl, triglycerides (TGs) ≥150 mg/dl, high-density lipoprotein cholesterol (HDL-C) <40 mg/dl, or those who were receiving drug treatment for dyslipidaemia. According to the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome[12], metabolic syndrome was defined as the presence of central obesity (waist circumference ≥85 cm in males and ≥90 cm in females) as well as two or more following risk factors: lipid abnormalities (TGs ≥150 mg/dl and/or HDL-C <40 mg/dl or use of medications for dyslipidaemia), high blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg or use of medications for hypertension), and hyperglycaemia (fasting plasma glucose ≥110 mg/dl or use of medications for diabetes mellitus). Smoking history was classified as never smokers, former smokers, and current smokers. Pack-year was calculated as the number of packs smoked per day multiplied by the number of years that subjects had smoked.

Statistical analyses

The statistical analyses were performed using JMP software for Windows, version 12 (SAS Institute Inc., Cary, NC, USA). The association between smoking status and NAFLD was evaluated using multivariate logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated following adjustment for age (≥40 years vs. ≤39 years), sex, alcohol consumption (light alcohol drinker vs. non-drinker), and metabolic syndrome (presence or absence). The association between the duration of smoking cessation and moderate to severe fatty liver in male former smokers was evaluated using multivariate logistic regression analysis adjusted by age (≥40 years vs. ≤39 years), alcohol consumption (light alcohol drinker vs. non-drinker), metabolic syndrome (presence or absence), and BI (≥400 vs. ≤399). Continuous variables are shown as the mean±standard deviation (SD). Comparisons of clinical characteristics among three groups were performed by one-way ANOVA for continuous variables or chi-square tests for categorical variables. A P value <0.05 was considered to be statistically significant.

RESULTS

Clinical characteristics of the study subjects stratified by smoking history

Clinical characteristics are summarized according to smoking history (Table I). The breakdown of the smoking history of the subjects is as follows: 9,720 (72.2%) never smokers, 2,023 (15.0%) former smokers, and 1,723 (12.8%) current smokers. The male subjects accounted for 4/5 of the subjects in both the former smokers and current smokers groups. The former smokers were older than the current smokers, and subjects with light drinking habits, hypertension and metabolic syndrome were more frequently observed in the former smokers group than in the current smokers group.

The proportions of subjects with NAFLD in the former smokers group and the current smokers group were 52.4% and 52.5%, respectively, which were significantly higher than that (33.0%) of the never smokers (P<0.0001). Additionally, the proportion of the subjects with moderate to severe fatty liver was significantly higher in both the former smokers and the current smokers groups than that in the never smokers group (P<0.0001), indicating that the severity of fatty liver was more advanced in both former smokers and the current smokers.

Table I. Comparisons of clinical characteristics of the study subjects stratified by smoking history

		Smoking history			
	Total subjects (n=13466)	**Never (n=9720)**	**Former (n=2023)**	**Current (n=1723)**	***P* value**
Mean age±SD (years)	53.0 ± 10.9	52.9 ± 11.1	55.4 ± 10.6	50.9 ± 9.8	< 0.0001
≤39 years	1400 (10.4%)	1059 (10.9%)	135 (6.7%)	206 (12.0%)	
40-59 years	8232 (61.1%)	5946 (61.2%)	1121 (55.4%)	1165 (67.6%)	
≥60 years	3834 (28.5%)	2715 (27.9%)	767 (37.9%)	352 (20.4%)	
Sex					< 0.0001
Male	6642 (49.3%)	3546 (36.5%)	1666 (82.4%)	1430 (83.0%)	
Female	6824 (50.7%)	6174 (63.5%)	357 (17.7%)	293 (17.0%)	
Alcohol consumption					< 0.0001
Non-drinker	6143 (45.6%)	4879 (50.2%)	628 (31.0%)	636 (36.9%)	
Light drinker	7323 (54.4%)	4841 (49.8%)	1395 (69.0%)	1087 (63.1%)	
BMI±SD (kg/m^2)	22.8 ± 3.5	22.5 ± 3.5	23.8 ± 3.4	23.8 ± 3.6	< 0.0001
Obesity (BMI ≥25 kg/m^2)					< 0.0001
Present	3132 (23.3%)	1988 (20.5%)	613 (30.3%)	531 (30.8%)	
Absent	10334 (76.7%)	7732 (79.5%)	1410 (69.7%)	1192 (69.2%)	
Diabetes mellitus					< 0.0001
Present	622 (4.6%)	353 (3.6%)	150 (7.4%)	119 (6.9%)	
Absent	12844 (95.4%)	9367 (96.4%)	1873 (92.6%)	1604 (93.1%)	
Hypertension					< 0.0001
Present	2506 (18.6%)	1697 (17.5%)	501 (24.8%)	308 (17.9%)	
Absent	10960 (81.4%)	8023 (82.5%)	1522 (75.2%)	1415 (82.1%)	
Dyslipidaemia					< 0.0001
Present	6094 (45.3%)	4113 (42.3%)	1025 (50.7%)	956 (55.5%)	
Absent	7372 (54.8%)	5607 (57.7%)	998 (49.3%)	767 (44.5%)	
Metabolic syndrome					< 0.0001
Present	1235 (9.2%)	668 (6.9%)	332 (16.4%)	235 (13.6%)	
Absent	12231 (90.8%)	9052 (93.1%)	1691 (83.6%)	1488 (86.4%)	
NAFLD					< 0.0001
Present	5169 (38.4%)	3204 (33.0%)	1060 (52.4%)	905 (52.5%)	
Moderate to severe fatty liver	2511 (18.7%)	1521 (15.7%)	513 (25.4%)	477 (27.7%)	
Absent	8297 (61.6%)	6516 (67.0%)	963 (47.6%)	818 (47.5%)	
Pack-year					< 0.0001
0	9720 (72.2%)	9720 (100%)	0 (0%)	0 (0%)	
0.01-9.99	912 (6.8%)	0 (0%)	625 (30.9%)	287 (16.7%)	
10.00-19.99	1001 (7.4%)	0 (0%)	528 (26.1%)	473 (27.5%)	
≥20.00	1833 (13.6%)	0 (0%)	870 (43.0%)	963 (55.9%)	

BMI: Body mass index; NAFLD: Non-alcoholic fatty liver disease; SD: Standard deviation.

Multivariate logistic regression analysis of the factors associated with NAFLD

Table II shows the results of the multivariate logistic regression analysis of the factors associated with NAFLD. The odds ratio was adjusted for age, sex, presence of metabolic syndrome, light alcohol consumption, and smoking history. Both former smoking and current smoking were significantly associated with NAFLD in the multivariate analysis (former smoking: OR 1.23, 95% CI 1.10-1.38; current smoking: OR 1.31, 95% CI 1.17-1.47). Metabolic syndrome (OR 9.24, 95% CI 7.79-11.0), male sex (OR 3.27, 95% CI 3.00-3.57), age ≥40 years (reference: ≤39 years) (OR 1.60, 95% CI 1.41-1.83), and light drinking (OR 0.77, 95% CI 0.71-0.84) were also significantly

associated with NAFLD. In addition, a significant positive association was observed in the multivariate analysis between current smoking (OR 1.29, 95% CI 1.13-1.48) and moderate to severe fatty liver.

Table II. Multivariate logistic regression analysis of the factors associated with NAFLD

	NAFLD		NAFLD (moderate to severe fatty liver)	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
Age ≥40 years (vs. ≤39 years)	1.60 (1.41-1.83)	< 0.0001	1.46 (1.22-1.74)	< 0.0001
Male sex (yes)	3.27 (3.00-3.57)	< 0.0001	2.75 (2.46-3.08)	< 0.0001
Light alcohol drinker (vs. non-drinker)	0.77 (0.71-0.84)	< 0.0001	0.65 (0.59-0.72)	< 0.0001
Metabolic syndrome (presence)	9.24 (7.79-11.0)	< 0.0001	7.60 (6.67-8.66)	< 0.0001
Smoking history				
Former smoker (vs. never smoker)	1.23 (1.10-1.38)	0.0002	1.05 (0.92-1.20)	0.49
Current smoker (vs. never smoker)	1.31 (1.17-1.47)	< 0.0001	1.29 (1.13-1.48)	0.0003

The odds ratio was adjusted for age, sex, presence of metabolic syndrome, light alcohol consumption, and smoking history. CI: Confidence interval; NAFLD: Non-alcoholic fatty liver disease; OR: odds ratio.

Association between smoking history stratified by pack-year and NAFLD

The association between smoking history stratified by the pack-year and NAFLD is shown in Table III. In the multivariate analysis, significant differences in the presence or absence of NAFLD were observed in the former smokers with a pack-year ≥20.00 (OR 1.40, 95% CI 1.19-1.64), the current smokers with a pack-year from 10.00 to 19.99 (OR 1.50, 95% CI 1.23-1.83), and the current smokers with a pack-year ≥20.00 (OR 1.40, 95% CI 1.20-1.63).

Association between smoking history and NAFLD based on the gender

Because smoking was strongly associated with NAFLD in males, the association of smoking status with pack-year and NAFLD was analysed stratified by gender. As the pack-year increased, the proportion of the subjects with moderate to severe fatty liver significantly increased irrespective of gender (Figure 1). As shown in Table III, multivariate analysis of the male subjects revealed that former smoking with a pack-year ≥20.00 (OR 1.44, 95% CI 1.22-1.72), current smoking with a pack-year from 10.00 to 19.99 (OR 1.54, 95% CI 1.23-1.94), and current smoking with a pack-year ≥20.00 (OR 1.43, 95% CI 1.22-1.68) were significantly associated with NAFLD. However, in the multivariate analysis of female subjects, a significant negative association between current smoking with a pack-year from 0.01 to 9.99 (OR 0.41, 95% CI 0.19-0.76) and NAFLD was observed.

Association between the duration of smoking cessation and severity of fatty liver in male former smokers

The clinical characteristics of the male former smoker subjects stratified by the duration of smoking cessation are shown in Table IV. The prevalence of moderate to severe fatty liver significantly decreased as the duration of smoking cessation increased (within 5 years, 117/345 [33.9%]; 6-10 years, 129/424 [30.4%]; more than 10 years, 227/897 [25.3%]; *P*=0.006). In the multivariate logistic regression analysis, a significant negative association was observed between the duration of smoking cessation (more than 10 years vs. within 5 years, OR 0.71, 95% CI 0.53-0.96) and moderate to severe fatty liver (Table V).

Table III. Multivariate logistic regression analysis to assess the association between smoking history and NAFLD

Smoking history	No NAFLD	NAFLD		
	n (%)	n (%)	OR (95% CI)	*P* value
Total (n=13466)	(n=8297)	(n=5169)		
Never	6516 (78.5)	3204 (62.0)	1 (reference)	
Former				
Pack-year 0.01-9.99	362 (4.4)	263 (5.1)	1.11 (0.93-1.33)	0.26
Pack-year 10.00-19.99	262 (3.2)	266 (5.1)	1.18 (0.98-1.43)	0.084
Pack-year ≥20.00	339 (4.1)	531 (10.3)	1.40 (1.19-1.64)	< 0.0001
Current				
Pack-year 0.01-9.99	192 (2.3)	95 (1.8)	0.84 (0.64-1.10)	0.21
Pack-year 10.00-19.99	227 (2.7)	246 (4.8)	1.50 (1.23-1.83)	< 0.0001
Pack-year ≥20.00	399 (4.8)	564 (10.9)	1.40 (1.20-1.63)	< 0.0001
Males (n=6642)	(n=3055)	(n=3587)		
Never	1761 (57.6)	1785 (49.8)	1 (reference)	
Former				
Pack-year 0.01-9.99	204 (6.7)	217 (6.0)	1.11 (0.90-1.37)	0.32
Pack-year 10.00-19.99	200 (6.5)	241 (6.7)	1.17 (0.95-1.44)	0.14
Pack-year ≥20.00	295 (9.7)	509 (14.2)	1.44 (1.22-1.72)	< 0.0001
Current				
Pack-year 0.01-9.99	100 (3.3)	85 (2.4)	0.96 (0.70-1.31)	0.80
Pack-year 10.00-19.99	151 (4.9)	216 (6.0)	1.54 (1.23-1.94)	0.0001
Pack-year ≥20.00	344 (11.3)	534 (14.9)	1.43 (1.22-1.68)	< 0.0001
Females (n=6824)	(n=5242)	(n=1582)		
Never	4755 (90.7)	1419 (89.7)	1 (reference)	
Former				
Pack-year 0.01-9.99	158 (3.0)	46 (2.9)	1.10 (0.77-1.55)	0.58
Pack-year 10.00-19.99	62 (1.2)	25 (1.6)	1.41 (0.86-2.23)	0.17
Pack-year ≥20.00	44 (0.8)	22 (1.4)	1.50 (0.85-2.55)	0.15
Current				
Pack-year 0.01-9.99	92 (1.8)	10 (0.6)	0.41 (0.19-0.76)	0.004
Pack-year 10.00-19.99	76 (1.4)	30 (1.9)	1.23 (0.77-1.90)	0.38
Pack-year ≥20.00	55 (1.0)	30 (1.9)	1.51 (0.92-2.44)	0.10

The odds ratio was adjusted for age, sex, presence of metabolic syndrome and light alcohol consumption.
BMI: Body mass index; CI: Confidence interval; NAFLD: Non-alcoholic fatty liver disease; OR: Odds ratio.

Table IV. Clinical characteristics of male former smokers stratified by the duration of smoking cessation

	Duration of smoking cessation			
	Within 5 years (n=345)	**6-10 years (n=424)**	**More than 10 years (n=897)**	***P* value**
Mean age±SD (years)	53.0 ± 10.6	54.2 ± 10.5	58.9 ± 9.6	< 0.0001
Age group				< 0.0001
≤39 years	32 (9.3%)	35 (8.3%)	16 (1.8%)	
40-59 years	208 (60.3%)	248 (58.5%)	436 (48.6%)	
≥60 years	105 (30.4%)	141 (33.3%)	445 (49.6%)	
Alcohol consumption (light drinker)	234 (67.8%)	308 (72.6%)	688 (76.7%)	0.005
BMI, mean±SD (kg/m^2)	24.4 ± 3.3	24.3 ± 3.1	23.9 ± 3.0	0.006
Obesity (BMI ≥25 kg/m^2)	125 (36.2%)	158 (37.3%)	266 (29.7%)	0.008
Hypertension (+)	75 (21.7%)	108 (25.5%)	283 (31.6%)	0.001
Dyslipidaemia (+)	189 (54.8%)	256 (60.4%)	461 (51.4%)	0.009
Diabetes mellitus (+)	24 (7.0%)	46 (10.9%)	71 (7.9%)	0.11
Metabolic syndrome (+)	70 (20.3%)	96 (22.6%)	155 (17.3%)	0.060
NAFLD (+)	213 (61.7%)	255 (60.1%)	499 (55.6%)	0.089
Moderate to severe fatty liver	117 (33.9%)	129 (30.4%)	227 (25.3%)	0.006
Pack-year, mean±SD	28.35 ± 22.13	26.62 ± 18.83	18.11 ± 17.46	< 0.0001
Pack-year category				< 0.0001
0.01-9.99	58 (16.8%)	71 (16.8%)	292 (32.6%)	
10.00-19.99	77 (22.3%)	92 (21.7%)	272 (30.3%)	
≥20.00	210 (60.9%)	261 (61.6%)	333 (37.1%)	

BMI: Body mass index; NAFLD: Non-alcoholic fatty liver disease; SD: Standard deviation.

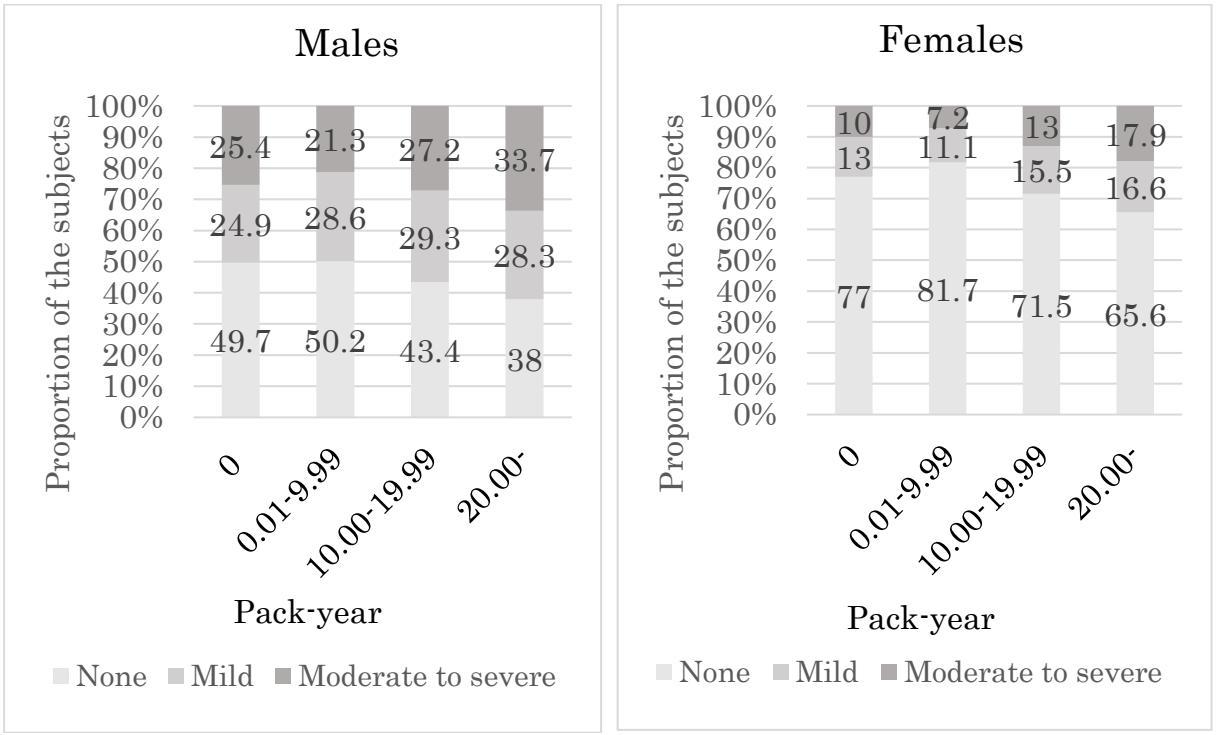

Figure 1. Association between the pack-year and the severity of fatty liver in males and females

Table V. Multivariate logistic regression analysis to assess the association between smoking cessation and NAFLD in males

	No NAFLD (moderate to severe fatty liver) (n=1193)	NAFLD (moderate to severe fatty liver) (n=473)		
			OR (95% CI)	*P* value
Duration of smoking cessation	n (%)	n (%)		
Within 5 years	228 (19.1)	117 (24.7)	1 (reference)	
6-10 years	295 (24.7)	129 (27.3)	0.81 (0.58-1.12)	0.20
More than 10 years	670 (56.2)	227 (48.0)	0.71 (0.53-0.96)	0.028

The odds ratio was adjusted for age, presence of metabolic syndrome, light alcohol consumption, and pack-year.
CI: Confidence interval; NAFLD: Non-alcoholic fatty liver disease; OR: Odds ratio.

DISCUSSION

In this study, not only former smoking but also current smoking was identified as a statistically significant factor associated with NAFLD. The statistical analysis of the study subjects stratified by smoking history and pack-year showed a significant association between smoking and NAFLD in the subjects with a high pack-year (former smokers with a pack-year ≥20.00, current smokers with a pack-year from 10.00 to 19.99 and a pack-year ≥20.00). Additionally, in this study, smoking history was associated with the severity of fatty liver diagnosed by ultrasonography, which was consistent with a previous report indicating that smoking history was associated with advanced liver fibrosis in NAFLD patients[13].

Although a meta-analysis study on the association between smoking and NAFLD did not reveal a significant association between current smoking and NAFLD[10], a significant association between current smoking with a high pack-year and NAFLD, which was observed in this study, has been reported in several previous studies. In a cross-sectional study of 2,691 Chinese male subjects in Shanghai, current heavy smokers (≥40 cigarettes/day) have been reported to have a 2.29-fold (95% CI 1.30-4.03) higher risk for NAFLD than non-smokers[11]. Additionally, in another cross-sectional study of 9,432 Chinese male subjects, compared to non-smokers, the risk ratios for NAFLD of the current 20-39.9 pack-year smokers and the current 40 or more pack-year smokers were reported to be 1.22 (95% CI 1.00-1.49) and 1.52 (95% CI 1.22-1.88), respectively[14]. In addition, in a study of 2,811 subjects based on the Rotterdam study, which was a large prospective cohort study conducted from 2009 to 2012, the pack-year smoking habits of former and current smokers were significantly associated with NAFLD (OR 1.01, 95% CI 1.00-1.01)[15]. Furthermore, urinary cotinine-verified current smoking was recently identified as an independent risk factor for NAFLD (OR 1.10, 95% CI 1.06-1.14) in a large cross-sectional study of 160,862 subjects based on the Kangbuk Samsug Health Study[16].

The relationship between current smoking and NAFLD has also been demonstrated in longitudinal studies. In a longitudinal study of 2,029 subjects who underwent medical health check-ups at 10-year intervals, Hamabe et al. reported that current smoking was a risk factor for the onset of NAFLD (OR 1.91, 95% CI 1.34-2.72), and the risk increased as the BI increased (BI 1-399: OR 1.77, 95% CI 1.02-3.07, BI ≥400: OR 2.04, 95% CI 1.37-3.03)[7]. Additionally, Okamoto et al. conducted a longitudinal study of 3,860 subjects who had undergone health check-ups and whose smoking habits and alcohol consumption were constant over the average observational period of 3.96 years and reported that current smoking was a risk factor for the onset of NAFLD in non-drinkers (OR 1.988, 95% CI 1.057-3.595)[8]. In addition, recently, a cohort study of 199,468 subjects followed for a median of 4.1 years, which was based on the Kangbuk Samsug Health Study, demonstrated that current smoking, pack-years, and urinary cotinine level were positively associated with the risk of incident NAFLD[9].

A significant association between former smoking and NAFLD, which was observed in our study, was shown in a recent meta-analysis study[10]. Post-smoking cessation weight gain in former smokers is presumed to be involved in the occurrence of NAFLD[10, 17]. Although it is unknown whether the weight of the former smoker subjects in this study had increased after smoking cessation, the prevalence of obesity in the former smoker subjects was higher than that in the never smoker subjects.

There are several animal model studies on the mechanisms of the association between smoking and NAFLD. Yuan et al. reported that second-hand smoke stimulates lipid accumulation in the mouse liver by modulating AMPK and SREBP-1[18]. Additionally, Azzalini et al. reported that smoking causes oxidative stress and worsens the severity of NAFLD in obese rats[19]. Regarding clinical data, smoking causes insulin resistance[20] and is associated with an increase in visceral fat[21, 22], which might lead to the occurrence of NAFLD.

Many reports have shown that the prevalence of NAFLD in females is lower than that in males[23-25], which is consistent with the results of this study. One of the reasons for the low prevalence of NAFLD in females is that oestrogens suppress the accumulation of visceral fat by regulating adipose tissue[26, 27], which might result in prevention of the development of NAFLD. Smoking is reported to be associated with high testosterone levels in postmenopausal women[28] and with an androgenic profile in young women[29]. The change in sex hormone levels induced by smoking might influence the accumulation of visceral fat[29] and the development of NAFLD in female subjects.

Although the influence of sex on the association between smoking status and NAFLD has not been fully elucidated, the results of this study suggest that a sex difference might exist. In male subjects, a statistically significant association was observed in the multivariate analysis of the former smokers with a pack-year ≥20.00, the current smokers with a pack-year from 10.00 to 19.99 and the current smokers with a pack-year ≥20.00. However, in female subjects, the current smokers with a pack-year from 0.01 to 9.99 was negatively associated with NAFLD. The lower prevalence of obesity in the female current smoker subjects (current smokers 7.8% vs never smokers 15.4%, *P*=0.0359, Supplementary Table I) is likely to be involved in the observed negative association. Regarding weight loss due to smoking, nicotine has been reported to reduce body weight by raising the resting metabolic rate and suppressing appetite[30]. Recently, Jung et al. conducted a longitudinal cohort study based on the Kangbuk Samsug Health Study and reported a positive association between current smoking and the incidence of NAFLD in both men and women[9]. However, they reported that in their cross-sectional design analysis, a positive association between current smoking and the prevalence of NAFLD was observed in men but not in women[9], which was consistent with our study results. NAFLD has been recently reported to be associated with increased overall mortality and death from cancer, cardiovascular disease, and liver disease in women[31]. Further research is needed on smoking as a cause of NAFLD in females.

In the male smoker subjects, a significant negative association was observed between the duration of smoking cessation and the prevalence of moderate to severe fatty liver. It was recently reported that a longer duration of smoking cessation resulted in a greater decrease in insulin resistance in asymptomatic Korean male former smokers[32]. It was suggested that smoking cessation might lead to improved NAFLD, especially in male patients.

The strength of this study is a large amount of data from more than 10,000 subjects from a cross-sectional survey. However, this study has several limitations. First, it is impossible to determine a causal relationship between smoking and NAFLD because this is a cross-sectional study. In addition, although there were 6,824 female subjects in this study, the number of female current smokers and former smokers were relatively small at 293 (4.3%) and 352 (5.2%), respectively, which might have influenced the results of the multivariate analysis stratified by smoking status with pack-year in female subjects.

CONCLUSIONS

In conclusion, not only former smoking but also current smoking was identified as a significant risk factor of NAFLD, and smoking cessation might decrease the severity of NAFLD in male subjects. In female subjects, light current smoking was negatively associated with NAFLD. Further prospective studies are needed to clarify the causal relationship between smoking and NAFLD and the sex difference of that relationship.

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Supplementary Table I. Clinical characteristics of the study subjects stratified by sex and smoking history

	Smoking history						
	Never	**Former**			**Current**		
		Pack-year 0.01-9.99	**Pack-year 10.00-19.99**	**Pack-year ≥20.00**	**Pack-year 0.01-9.99**	**Pack-year 10.00-19.99**	**Pack-year ≥20.00**
Male	(n=3546)	(n=421)	(n=441)	(n=804)	(n=185)	(n=367)	(n=878)
Mean age ± SD (years)	53.1 ± 11.5	50.9 ± 10.6	54.4 ± 10.0	60.5 ± 8.7	43.2 ± 10.8	47.1 ± 8.5	54.5 ± 8.5
≤39 years	404 (11.4%)	61 (14.5%)	19 (4.3%)	3 (0.4%)	86 (46.5%)	72 (19.6%)	13 (1.5%)
40-59 years	2094 (59.1%)	264 (62.7%)	267 (60.5%)	361 (44.9%)	87 (47.0%)	263 (71.7%)	607 (69.1%)
≥60 years	1048 (29.6%)	96 (22.8%)	155 (35.2%)	440 (54.7%)	12 (6.5%)	32 (8.7%)	258 (29.4%)
Light alcohol drinker	2380 (67.1%)	336 (79.8%)	332 (75.3%)	562 (69.9%)	140 (75.7%)	243 (66.2%)	556 (67.3%)
BMI, mean ± SD (kg/m^2)	23.7 ± 3.2	23.7 ± 3.1	23.9 ± 3.2	24.4 ± 3.0	23.5 ± 3.2	24.0 ± 3.6	24.2 ± 3.5
Obesity (BMI ≥25 kg/m^2)	1039 (24.3%)	121 (28.7%)	124 (28.1%)	304 (37.8%)	48 (26.0%)	118 (32.2%)	310 (35.3%)
Hypertension (+)	823 (23.2%)	81 (19.2%)	107 (24.3%)	278 (34.6%)	25 (13.5%)	51 (13.9%)	198 (22.6%)
Dyslipidaemia (+)	1717 (48.4%)	192 (45.6%)	231 (52.4%)	483 (60.1%)	74 (40.0%)	211 (57.5%)	563 (64.1%)
Diabetes mellitus (+)	201 (5.7%)	18 (4.3%)	24 (5.4%)	99 (12.3%)	7 (3.8%)	13 (3.5%)	92 (10.5%)
Metabolic syndrome (+)	484 (13.7%)	50 (11.9%)	67 (15.2%)	204 (25.4%)	19 (10.3%)	39 (10.6%)	160 (18.2%)
NAFLD (+)	1785 (50.3%)	217 (51.5%)	241 (54.7%)	509 (63.3%)	85 (46.0%)	216 (58.9%)	534 (60.8%)
Moderate to severe fatty liver	902 (25.4%)	91 (21.6%)	111 (25.2%)	271 (33.7%)	38 (20.5%)	109 (29.7%)	296 (33.7%)
Female	(n=6174)	(n=204)	(n=87)	(n=66)	(n=102)	(n=106)	(n=85)
Mean age ± SD (years)	52.7 ± 10.9	48.0 ± 9.8	51.8 ± 9.4	57.2 ± 10.2	45.2 ± 8.5	50.7 ± 8.8	53.8 ± 8.5
≤39 years	655 (10.6%)	44 (21.6%)	7 (8.1%)	1 (1.5%)	27 (26.5%)	6 (5.7%)	2 (2.4%)
40-59 years	3852 (62.4%)	130 (63.7%)	62 (71.3%)	37 (56.1%)	68 (66.7%)	81 (76.4%)	59 (69.4%)
≥60 years	1667 (27.0%)	30 (14.7%)	18 (20.7%)	28 (42.4%)	7 (6.9%)	19 (17.9%)	24 (28.2%)
Light alcohol drinker	2461 (39.9%)	109 (53.4%)	30 (34.5%)	26 (39.4%)	56 (54.9%)	49 (46.2%)	43 (50.6%)
BMI, mean ± SD (kg/m^2)	21.8 ± 3.4	22.0 ± 4.3	22.4 ± 3.9	22.5 ± 5.0	21.3 ± 3.5	22.6 ± 3.7	23.4 ± 4.6
Obesity (BMI ≥25 kg/m^2)	949 (15.4%)	33 (16.2%)	17 (19.5%)	14 (21.2%)	8 (7.8%)	23 (21.7%)	24 (28.2%)
Hypertension (+)	874 (14.2%)	15 (7.4%)	12 (13.8%)	8 (12.1%)	4 (3.9%)	13 (12.3%)	17 (20.0%)
Dyslipidaemia (+)	2396 (38.8%)	58 (28.4%)	29 (33.3%)	32 (48.5%)	23 (22.6%)	41 (38.7%)	44 (51.8%)
Diabetes mellitus (+)	152 (2.5%)	3 (1.5%)	5 (5.8%)	1 (1.5%)	1 (1.0%)	4 (3.8%)	2 (2.4%)
Metabolic syndrome (+)	184 (3.0%)	6 (2.9%)	1 (1.2%)	4 (6.1%)	2 (2.0%)	6 (5.7%)	9 (10.6%)
NAFLD (+)	1419 (23.0%)	46 (22.5%)	25 (28.4%)	22 (33.3%)	10 (9.8%)	30 (28.3%)	30 (35.3%)
Moderate to severe fatty liver	619 (10.0%)	17 (8.3%)	12 (13.8%)	11 (16.7%)	5 (4.9%)	13 (12.3%)	16 (18.8%)

BMI, body mass index; NAFLD, non-alcoholic fatty liver disease; SD, standard deviation